Understanding the neural repair promoting properties of olfactory ensheathing cells: Towards a treatment for spinal cord injuries

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1. Summary

The mammalian central nervous system has only a limited capacity to regenerate after damage which usually leads to a permanent loss of function. An exception is formed by the olfactory nervous system, which can fully recover after injury. The olfactory system contains continuous regenerating new olfactory neurons in the olfactory epithelium and olfactory ensheathing glia cells (OECs) that form a supportive cellular substrate for growing axons. Implantation of OECs has been shown to enhance neural repair in spinal cord, brain and peripheral nerve and in animal models for neurodegenerative diseases like amyotrophic lateral sclerosis or Parkinson's disease. OECs implants do promote axonal outgrowth and neuronal survival, stimulate angiogenesis and wound repair by modulating the immune response and removing cellular debris and OECs can myelinate regenerating axons (reviewed in **Chapter 1**). However, the outcome of OEC implantation studies has varied between laboratories and different pro-regenerative molecular profiles of OECs were reported, dependent on the source, age or purification methods of the cells. Moreover, several studies reported limited survival of OECs after implantation.

A comprehensive understanding of the molecular mechanisms that govern the survival and neural repair promoting properties of OECs can potentially be used to acquire new molecular insights into successful regeneration, enhance the therapeutic potential of OECs and to transfer their pro-regenerative properties to other cell types/ tissues. The primary aim of the work described in this thesis was to identify and study potential molecular components that underlie the neural repair promoting properties of OECs.

We conducted a multi-step screening approach, aimed at discovering proteins expressed by OECs that are involved in the stimulation of axonal outgrowth. This is described in **Chapter 2**. Two gene expression profiling studies investigating OECs in culture and in the regenerating olfactory nerve layer were used to select 106 candidate genes which were subsequently functionally validated with gain and loss of function cellular bioassays. This resulted in the identification of twenty proteins that promote neurite outgrowth of primary dorsal root ganglion neurons. Three of these proteins, S100A9, SCARB2 and SERPINI1 were selected for ex-vivo gene delivery in the lesioned rat dorsal column. In-vivo overexpression of one of these proteins, scavenger receptor class B, member 2, a lyosomal protein that is involved in peripheral nerve myelination, cholesterol metabolism and beta-glucocerebrosidase transport, resulted in enhanced regeneration of dorsal column fibers. This is the first report of the involvement of SCARB2 in neural repair.

In Chapter 3, a meta-analysis was executed on the five published microarray datasets investigating cultured OECs. The aims of this meta-analysis were

(i) to identify genes that are consistently detected in multiple microarray studies (instead of only a single study) and that therefore may play a key role in OEC-mediated neural repair and (ii) to generate new hypotheses about the function of OECs. The meta-analysis resulted in a "Top" list of 455 genes that were uniformly expressed in OECs in the majority of datasets. A gene ontology analysis on these 455 genes showed overrepresentation of genes that are part of molecular pathways involved in neurite outgrowth, blood vessel development, cell migration and modulation of the immune response. A molecular interaction network analysis revealed 21 Hub genes that are likely to function as modulators of these processes. Six genes were identified that were higher expressed in OECs in all datasets. After performing the meta-analysis we moved on, in **Chapter 4**, to demonstrate that ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2), the highest expressed gene in the "Top" list, is indeed highly expressed in cultured OECs an in OECs-implanted in the lesioned rat spinal cord. ENTPD2 is expressed on the surface of OECs and can hydrolyze highly toxic extracellular ATP. Since ATP is released from dying cells after a spinal cord lesion we hypothesize that the ecto-ATPase activity of OEC may partially explain the neuroprotective and analgesic effects of OEC implants.

OECs usually do not survive well after implantation in the lesioned spinal cord. A better survival rate would probably results in a more lasting positive impact of OEC-mediated neural repair. In **Chapter 5**, we demonstrated for the first time that in vivo bioluminescence imaging can be used for non invasive monitoring of implanted OECs and SCs in the lesioned rat dorsal column for up to three months. The bioluminescent signal showed a strong overall correlation with a classical histological analysis of the transplanted cells as determined at two post-implantation time-points. In vivo bioluminescence imaging is a powerful technique that can be used to assess the survival of OECs and SCs in the lesioned spinal cord without sacrificing the animals and conducting a time-consuming classical histological analysis. This would facilitate the analysis of the effectiveness of treatments that have the potential to improve the survival of transplanted cells in a non-invasive and longitudinal manner.

After implantation in the lesioned spinal cord, OECs intermingle better with the astrocytes and meningeal cells (MC) in the scar than SCs. In **Chapter 6**, we showed that in co-culture assays, knockdown of neuropilin-2 (NRP2) in SCs decreased MC induced aggregation of SCs and increased the number of loosely aligned SCs, while a knockdown of neuropilin-1 (NRP1) increased aggregate size. Interference with NRP1 and/or NRP2 function in SCs can potentially improve SC integration in the lesioned spinal cord, thereby making the scar more permeable for growing axons.